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Lipid rich carcinoma and solid carcinoma in mammary gland of a dog: histopathologic and immunohistochemical features

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Abstract

Lipid rich carcinoma is rare in dog and women and is characterized by cells that have an abundant foamy cytoplasm. A 10 year old, mix breed female dog suffered two subcutaneous masses in ventral abdomen was referred to surgery department of veterinary hospital of Ahvaz Shahid Chamran University. Surgical excision and microscopic examination of masses were done. After grading by Elston and Ellis method, histochemical staining was performed such as PAS, Cong red and Oil Red O. Immunohistochemical detection of pKi67, P53, c-erbB2 and factor VIII – related antigen were carried out. Microscopically, the masses were two parts; first compose of big cells with vacuolated cytoplasm and euchromatin nuclei and second part cells with scant cytoplasm and pleomorphic nuclei and obvious membrane. Grade of tumor was Grade III, poorly differentiated. Positive reaction was seen in vacuolated cytoplasm for Oil Red O. Neoplastic cells of lipid rich area demonstrated intense immunoreactivity for pKi67. All tumor cells lacked p53 and c-erbB2. This is the first report of coincidence of lipid rich carcinoma and solid carcinoma in a dog with description of histopathologic and immunohistochemical characteristics.

Keywords: Lipid rich carcinoma; solid carcinoma; dog; mammary gland; p53; c-erbB2.

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Introduction

Lipid rich carcinoma is rare variant of breast cancer with an aggressive clinical course and poor prognosis (Misdorp, *et al.* 1999; Misdorp, 2002; Shi *et al.,* 2008). This tumor which is resemble to sebaceous breast cancer has been reported in human, dog, cat and hamster (Espinosa De Los Monteros, *et al.,* 2003; Pena, *et al.,* 2003; Kamstock, *et al.,* 2005; Perez – Martinez, *et al.,* 2005; Chen, *et al.,* 2007 and Yoshimura, *et al.,* 2010) and it presents only 1 – 2 % of all breast cancers in woman (Shi et al 2008). Lipid rich carcinoma has been included in the most recent WHO's classification for mammary gland tumors of the dog and it is extremely rare in the species with low description for immunohistochemical characteristics (Misdorp, *et al.* 1999 and Misdorp, 2002). The aim of this report was to explore histopathologic and immunohistochemical features of lipid rich carcinoma with solid carcinoma.

Case presentation

A 10 year old female dog was referred to surgery department of veterinary hospital for two masses on the ventral abdomen, with three big ulcers 2.5 cm diameter.

The surgery carried out for masses removal. The masses were submitted to the pathology department for routine histopathologic examination. After fixation in 10% neutral buffered formalin, the tissue processed by common paraffin technique. Histopathological diagnosis was performed on the slides stained with haematoxylin and eosin. Grading was done according to Elston and Ellis methods which were described in detail elsewhere (Rezaie et al, 2009). Periodic acid – Shiff (PAS), Cogo red and Oil Red O as additional histochemical staining was performed. Oil Red O stain was performed on unprocessed formalin fixed frozen tissues.

Sections were also stained immunohistochemically for detection of pKi67, p53 protein, c-erbB2 and factor VIII – related antigen. Immunohistochemical method was described in detail elsewhere but briefly was as follow: histologic sections (5 µm) of the tumor was deparaffinized and rehydrated in graded ethanol. Antigens were retrieved by steaming in Dako retrieving solution (Dako, Denmark). Endogenous peroxidase activity was blocked by hydrogen peroxide with 1:10 dilution in methanol and for nonspecific binding, bovine serum albumin was used. Slides were incubated for primary antibodies: MIB1, Do-7, her2 and factor VIII – related antigen (Dakocytomation, Denmark). Slides were then incubated with biotinylated secondary antibodies and streptavidin - biotin peroxidase complex. The chromogen was 3-3 diaminobenzidine (DAB). The sections were counterstained in hematoxyline and fast green and then coverslipped. Human breast cancer sections were used as positive controls and negative controls were treated with PBSS as primary antibodies (Rezaie, *et al.,* 2010).

Results

Grossly, Surface of ulcers was light red and the borders were swollen. The cut surface of masses had greasy content.

Histopathologic examination revealed two parts, one include clusters of tumoric cells with foamy and vacuolated cytoplasm. The cells formed big acinies or cell sheets. The lumen of acini was filled by necrotic debris. Most of cells had distinct and round vacuoles. Nuclei had variably sized, predominantly euchromatin and basophilic nucleoli (Figure 1). These clusters were surrounded by thick connective tissue layer and they were beneath dermis. Ulcers were covered by necrotic debris and inflammatory cells especially neutrophils. Accumulations of plasma cells were seen between clusters or dense connective tissues. Part two composed of cells with scant eosinophilic cytoplasm and pleomorphic nuclei and prominent nucleoli which were located on basal lamina in multiple layers (Figure 2).

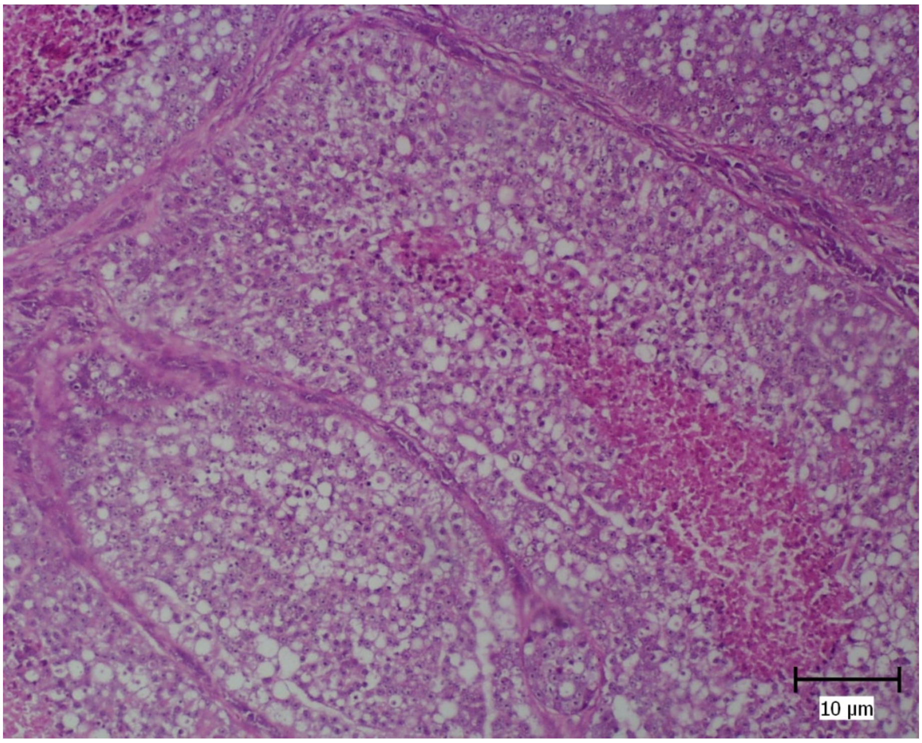


Figure 1: lipid rich carcinoma in a dog, showing clear and round vacuoles in cells (H&E).

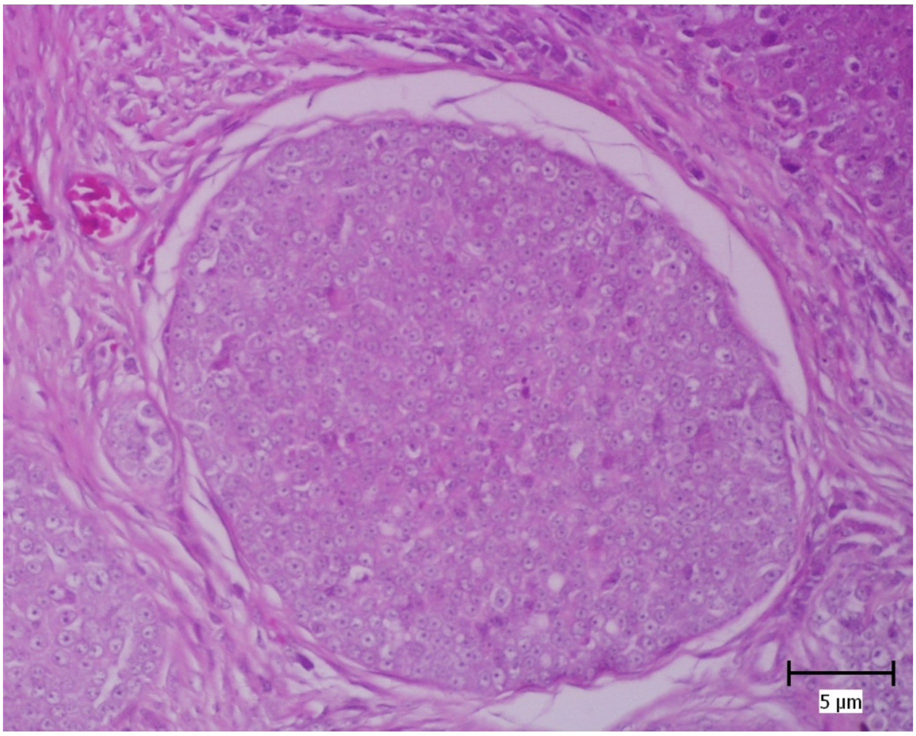


Figure 2: Solid carcinoma. Note large vesicular nuclei with prominent nucleoli (H&E).

The grade of this tumor was III, poorly differentiated. This method was briefly conducted on three parameters; tubule formation, nuclear pleomorphism and mitotic counts.

A histochemical result of PAS was basement membrane staining and Congo red result was negative and no particle detected. Cytoplsmic vacuoles of neoplastic cells were positive for Oil Red O and they were red in blue background.

Immunohistochemical staining results were as follow: pKi67 expression was detected by brown nuclei in both part of tumor; lipid rich area and solid area but positive cells were more in lipid rich area (Figure 3). P53 and c-erbB2 expression was negative. Rate of angiogenesis was assessed by factor VIII – related antigen. The internal surfaces of vessels were mottled dark brown and most endothelial cells expressed a granular to sometimes homogeneous precipitate of the chromogen in their cytoplasm, indicating they contained factor VIII – related antigen. There was no specific staining and the type of color and its distribution was different so detection from positive area was easy (Figure 4).

Discussion

Lipid rich carcinoma is one of the mammary gland carcinoma, characterized by cells with abundant vacuolated cytoplasm that contain a large amount of neutral lipid. Solid carcinoma is composing of diffuse cells. Lipid rich carcinoma which is recently documented in dogs is resemble to mammary carcinoma with sebaceous differentiation (Espinosa De Los Monteros, *et al.,* 2003; Pena, *et al.,* 2003 and Perez – Martinez, *et al.,* 2005). Cytoplasmic vacuoles in lipid rich carcinoma and mammary carcinoma with sebaceous differentiation which are positive in Oil Red O and negative in PAS have lipid entity but these lipid vacuoles in sebaceous differentiation produce scalloping or rounded indentation in the nuclear membrane that are different from those found in lipid rich carcinoma (Chang, *et al.,* 2007).

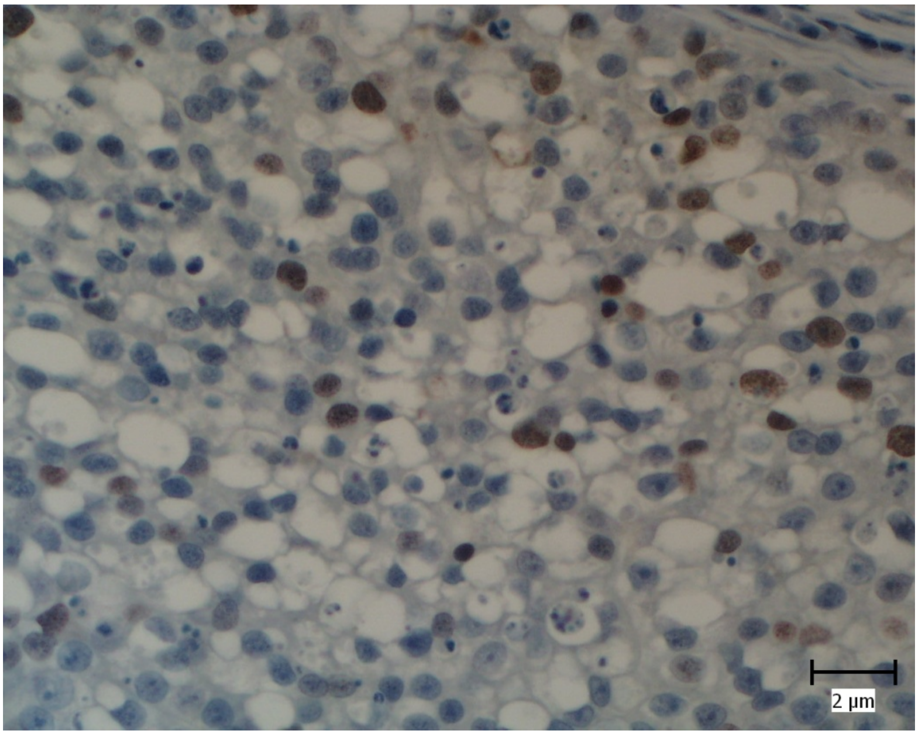


Figure 3: Lipid rich carcinoma. Brown nuclei are explanatory for pKi67 expression. (MIB-1 immunostaining, haematoxylin counterstain).

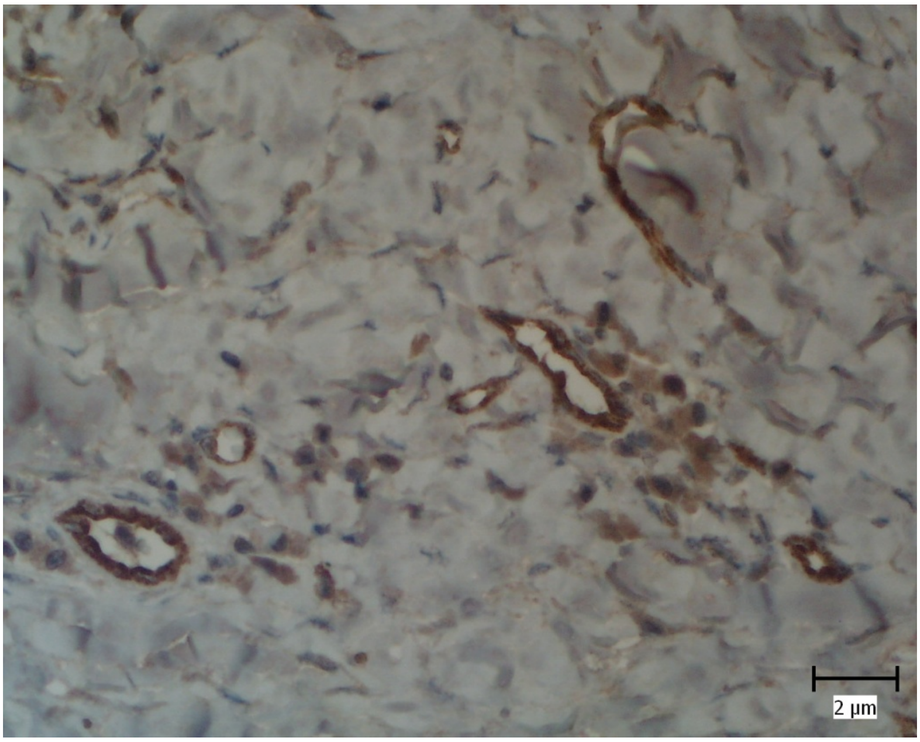


Figure 4: Factor VIII – related antigen expression. Note the distribution of brown foci which are showing the angiogenesis in the connective tissue around the tumor cells (Factor VIII – related antigen immunostaining, haematoxylin counterstain).

In this case the cells formed big acinies and there was a coincidence of lipid rich carcinoma and solid carcinoma. Pena et al also described lipid rich carcinoma with tubular disposition (Pena, *et al,* 2003). Espinosa de los monteros *et al* described seven cases of lipid rich carcinoma. Their results showed negative myoepithelial cell markers and so it was determined that the majority of neoplastic cells were of glandular epithelial origin (Espinosa De Los Monteros, *et al.,* 2003). The tubuloacinar pattern of the neoplastic cells in different reports support that lipid rich carcinoma may be originated from simple carcinoma.

The tumor proliferation measured by pKi67 immunostaing and it is in accordance with grade of tumor which is grade III, poorly differentiated. Six months after complete surgical excision of the mass, the tumor recurrence was diagnosed and due to bad condition the dog euthanatized without necropsy (due to owner idea). This marker considered a good prognostic factor in canine mammary tumors (Rezaie, *et al.,* 2011).

Immunohistochemical detection of p53 protein was negative. Yoshimura *et al* which was reported lipid rich carcinoma in a hamster, examined different markers such as p63 and the expression of p63 was negative. P53 and p63 are members of tumor suppressor genes. The p63 gene was discovered 20 years after the discovery of the p53 tumor suppressor gene and along with p73 constitutes the p53 gene family based on their structural similarity (Yoshimura, *et al.,* 2010).

Expression of c-erbB2 was negative which is in agreement with Chen *et al* results in human lipid rich carcinoma (Chen, *et al.,* 2007). Although Shi *et al* reported 71.4% positive c-erbB2 in 49 cases of human lipid rich carcinoma (Shi, *et al.,* 2008).

Factor VIII – related antigen is one of several components of the factor VIII glycoprotein complex which is present in plasma and plays an essential role in blood

coagulation. The presence of factor VIII – related antigen in this report is applicable for further diagnosis in canine mammary carcinoma and could provide better prognostic information for the veterinary clinician (Luong, *et al.*, 2006).

The findings from this case suggest that lipid rich carcinoma should be considered as subclassification of simple carcinoma.

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بررسی هیستوپاتولوژیک و ایمونوهیستوشیمیایی یک مورد کارسینوم غنی از چربی و کارسینوم توپر به‌طور همزمان در غدد پستانی سگ

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چکیده

کارسینوم غنی از چربی یکی از تومورهای نادر در انسان و سگ می‌باشد و مهم‌ترین مشخصه آن سلول‌های حاوی سیتوپلاسمی کف‌آلود می‌باشد. یک سگ ماده ۱۰ ساله با نژاد مختلط که از دو توده در ناحیه شکم زجر می‌برد به بخش جراحی بیمارستان دامپزشکی دانشگاه شهید چمران اهواز ارجاع داده شد. توده‌ها توسط عمل جراحی برداشت گردیدند و مورد بررسی دقیق میکروسکوپیک قرار گرفتند. پس از درجه‌بندی بر اساس روش Elston و Ellis رنگ‌آمیزی هیستوشیمیایی PAS، Congo Red و Oil Red O صورت گرفت. رنگ‌آمیزی ایمونوهیستوشیمیایی جهت ردیابی آنتی‌ژن‌های PKi67، P53، C-erbB2 و فاکتور هشت انجام گردید. در بررسی میکروسکوپیک، توده‌ها از دو قسمت مجزا تشکیل شدند. قسمت اول شامل سلول‌های بزرگ با سیتوپلاسم واکوئله و هسته روشن بوده و قسمت دوم از سلول‌هایی با حداقل سیتوپلاسم و هسته‌های پلئومورف و غشاء واضح تشکیل شدند. درجه تومور، درجه سه، تفکیک و تمایز نیافته بود. سلول‌های واکوئله در رنگ‌آمیزی Oil red O مثبت بودند. سلول‌های توموری در قسمت غنی از چربی واکنش قوی با آنتی‌بادی Ki67 را نشان دادند و درپاسخ با آنتی‌بادی P53 و C-erbB2 منفی بودند. بر اساس جستجوهای صورت گرفته این اولین مورد وقوع همزمان کارسینوم غنی از چربی و کارسینوم توپر در سگ می‌باشد.

واژگان کلیدی: کارسینوم غنی از چربی، کارسینوم توپر، سگ، غده پستانی، P53 ، C-erbB2